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The 75-Kilodalton Antigen of *Bartonella bacilliformis* Is a Structural Homolog of the Cell Division Protein FtsZ

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A genomic library of *Bartonella bacilliformis* was constructed and screened with human anti-*Bartonella* serum from a patient with the chronic, verruga peruana phase of bartonellosis. An immunoreactive clone isolated from this library was found to code for a 591-amino-acid protein with a high degree of sequence similarity to the FtsZ family of proteins. The degree of amino acid identity between the *B. bacilliformis* protein ($FtsZ_{Bb}$) and the other FtsZ proteins is especially pronounced over the N-terminal 321 amino acids (N-terminal domain) of the sequence, with values ranging from 45% identity for the homolog from *Micrococcus luteus* ($FtsZ_{Ml}$) to 91% identity for the homolog from *Rhizobium melliloti*, ($FtsZ_{Rm1}$). All of the functional domains required for FtsZ activity are conserved in $FtsZ_{Bb}$ and are located within the N-terminal domain of the protein. $FtsZ_{Bb}$ is approximately twice as large as most of the other FtsZ proteins previously reported, a property it shares with $FtsZ_{Rm1}$. Like the *Rhizobium* homolog, $FtsZ_{Bb}$ has a C-terminal region of approximately 256 amino acids that is absent in the other FtsZ proteins. Evidence is presented that implicates this region in the protein's antigenicity and suggests that, unlike most other FtsZ homologs, $FtsZ_{Bb}$ is at least partly exposed at the cell surface. PCR analysis revealed that an *ftsZ* gene similar in size to the *B. bacilliformis* gene is present in *Bartonella henselae*, a bacterium that is closely related to *B. bacilliformis*.

Bartonella bacilliformis is the etiologic agent of bartonellosis (Carrion's disease), a biphasic disease that is endemic among inhabitants of the western slopes of the Andes in Columbia, Ecuador, and Peru (11, 14). The disease consists of a primary, acute, hematic phase characterized by fever, hemolytic anemia, and bacteremia, referred to as Oroya fever. This is followed by a secondary, chronic phase characterized by skin eruptions, referred to as verruga peruana. During the hematic phase, *B. bacilliformis* parasitizes almost 100% of the erythrocytes, resulting in their premature destruction, and leading to a very severe form of hemolytic anemia. In the secondary phase, *B. bacilliformis* invades endothelial cells, causing hemangiomas, possibly as a result of hyperproliferation of the terminal vasculature of the dermis and subcutaneous tissue (10). These hemangiomas bear a distinct clinical and histologic similarity to the hemangioma-like lesions of bacillary (epitheliod) angiomatosis (BA), which has now been associated with the newly described bacterium *Bartonella henselae* (previously, *Rochalimaea henselae*) (4, 26, 27). Interestingly, *B. bacilliformis* and *B. henselae* are also closely related phylogenetically, and it was suggested that these bacteria may have a common mechanism of pathogenesis (1).

We were interested in isolating and characterizing immunodominant *B. bacilliformis* antigens because of their potential usefulness in diagnosis and in elucidating the pathogenesis of bartonellosis. Additionally, the presence of homologs of these antigens in *B. henselae* might be interesting from the standpoint of the pathological similarities between the lesions of verruga peruana and BA. To isolate immunodominant antigens of *B. bacilliformis*, we constructed a *B. bacilliformis* genomic DNA library and screened it with serum from a patient with the chronic verruga phase of bartonellosis. In this report, we describe the isolation and initial characterization of one of the clones expressing a 75-kDa antigen of *B. bacilliformis*. Based on the strong amino acid sequence homology with the cell division protein FtsZ, we suggest that the 75-kDa antigen is the *B. bacilliformis* homolog of FtsZ ($FtsZ_{Bb}$). This is the first study that shows that FtsZ proteins are immunogenic in humans.

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MATERIALS AND METHODS

Bacterial strains, plasmids, media, and serum. *B. bacilliformis* KC584 (ATCC 35686) and KC583 (ATCC 35685) were grown on heart infusion agar plates supplemented with 5% defibrinated rabbit blood (BBL) at 28°C for 6 to 8 days. Bacteria were harvested and resuspended in phosphate-buffered saline. The cells were then collected by centrifugation at 8,000 × *g*, and the supernatant was discarded. The pellets were then resuspended in TE (10 mM Tris [pH 8.0], 1 mM EDTA) for DNA extraction.

The XL-1*Blue* strain of *Escherichia coli* (Stratagene, Torrey Pines, La Jolla, Calif.) was grown on Luria broth (LB) with tetracycline (12.5 μg/ml) or LB with ampicillin (50 μg/ml) when transformed with pBluescript (Stratagene). *E. coli* SOLR cells were grown on LB with kanamycin (50 μg/ml).

The human serum was obtained from a 14-year-old Ecuadorian patient with the verruga peruana phase of bartonellosis. The serum had a high antibody titer of 1:512 as tested by indirect fluorescent-antibody assay at the Centers for Disease Control and Prevention, Atlanta, Ga.

pKKORF was constructed by cloning ORF-591 into pKK223-3. ORF-591 was amplified by using the PCR. Primers used in the amplification were 5′ AAAG AATTCTCGACAGGTAGAGGTAAGTAT 3′ and 5′ TAACCCGGGATACA TTGCCACTATGAACAC 3′, which incorporated *Eco*RI and *Xma*I sites, respectively (underlined). The amplified fragment was digested and ligated to pKK223-3 that was cleaved with *Eco*RI and *Xma*I. The usual cloning procedures were followed (28).

DNA extraction. Total genomic DNA was extracted from strain KC584 by a protocol described earlier (1). Plasmid DNA for sequencing was prepared by cesium chloride gradient purification (28).

Agarose gel electrophoresis. Genomic DNA extracted from cells, as well as restriction digests were electrophoresed through a 0.7% agarose gel. Gels were electrophoresed in Tris-borate buffer containing ethidium bromide and photographed. *Tsp*509I fragments ranging in size from 3 to 8 kb were extracted from agarose gels using the GENECLEAN (BIO 101 Inc., La Jolla, Calif.) protocol.

Construction of *B. bacilliformis* genomic library. Total genomic DNA from *B. bacilliformis* KC584 was partially digested with the restriction endonuclease *Tsp*509I (New England Biolabs) (65°C for 30 min). Fragments in the size range of 3 to 8 kb were ligated to *Eco*RI-digested, alkaline phosphatase-treated lambda ZAPII vector arms, using the lambda ZAPII cloning system (Stratagene). The ligation products were packaged in vitro using the GIGAPACK II GOLD packaging system (Stratagene). Packaged lambda phage was then used to infect *E. coli* XL-1*Blue*. The titer of the resulting library was approximately 8×10^8 recombinant phage/ml.

Library screening. Approximately 50,000 recombinant lambda phage were screened for the production of recombinant proteins that were specific for *B. bacilliformis*. Filters were blocked overnight with 5% BLOTTO and reacted with the primary antibody, human *B. bacilliformis* antiserum (diluted 1:200 in 5% BLOTTO), for 2 h using gentle agitation (approximately 80 rpm). The secondary antibody was goat anti-human immunoglobulin G conjugated to horseradish peroxidase, diluted 1:5,000 in 5% BLOTTO (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md.). Filters were developed using a standard chromogenic substrate (TMB membrane peroxidase substrate system; Kirkegaard and Perry Laboratories Inc.). Positive plaques were picked and purified by plating at successively lower densities. Immunopositive plaques from the final round of purification were subcloned by using the lambda ZAPII in vivo excision method (ExAssist/SOLR system; Stratagene).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. *E. coli* harboring the recombinant plasmids (from the immunopositive plaques) as well as an *E. coli* negative control harboring pBluescript were induced with isopropyl-β-D-thiogalactopyranoside (1 mM) to express proteins. Proteins from the bacterial preparations were then solubilized in 1× sample buffer (15) at 100°C and electrophoresed on precast 8 to 16% polyacrylamide gradient gels (NOVEX). Gels were run using Tris-glycine-SDS running buffer. Separated proteins were either stained with Coomassie brilliant blue or electrotransferred onto 0.45-μm-pore-size nitrocellulose membranes according to the method of Towbin et al. (32). The transfer buffer was Tris-glycine with 20% methanol (NOVEX). Transfer was done for 2 h at 100 V with cooling. Membranes were blocked overnight in 5% BLOTTO. The blotting protocol was essentially as described for the nitrocellulose filters, under "Library screening." Membranes were reacted with either human anti-*Bartonella* serum or *E. coli* anti-FtsZ serum, and the appropriate secondary antibody (anti-human or anti-rabbit immunoglobulin G) was used.

DNA sequencing. The nucleotide sequence of both strands of the pIN1 insert was obtained by the methods of Sanger et al. (29) using a model 373A automated nucleic acid sequencer (Applied Biosystems, Foster City, Calif.) and *fmol* cycle sequencing (Promega). In addition to the T3 and T7 primers, internal sequencing primers were synthesized by using a model 394 DNA synthesizer (Applied Biosystems).

DNA sequence analysis. DNA and protein sequence analyses were performed by using the software packages MacDNAsis (Hitachi Software Engineering America, Ltd., San Bruno, Calif.) and MacVector (International Biotechnologies, Inc., New Haven, Conn.). Multiple sequence alignments were done using the Higgins-Sharp algorithm (Clustal4) of MacDNAsis.

Partial purification of $FtsZ_{Bb}$. Cells expressing the protein were grown up in a large volume (6 liters) of LB containing ampicillin and induced for protein expression. The culture was concentrated to 1 liter by using a Millipore concentrator (Pellicon; Millipore Corp., Bedford, Mass.). Cells were pelleted, washed once in resuspension buffer (40 mM Tris-HCl [pH 8.0], 5 mM EDTA, 140 mM NaCl, 1 mM dithiothreitol, 10% sucrose, 200 μg of phenylmethylsulfonyl fluoride per ml, and COMPLETE protease inhibitor cocktail mix [Boehringer Mannheim], and resuspended in the same buffer (2 ml/g of cells). Resuspended cells were broken by using a French cell press (SLM AMINCO; SLM Instruments, Inc.) by applying a pressure of 8,000 lb/in^2. The cell lysate was centrifuged at 27,000 × *g* for 30 min at 4°C in a Beckman model J2-MI ultracentrifuge (Beckman Instruments, Palo Alto, Calif.) using a JA-20 rotor. The supernatant was transferred to a clean tube, and 20% streptomycin sulfate was added, mixed, equilibrated at 0°C for 10 min, and centrifuged as before. Proteins in the supernatant were precipitated by adding ammonium sulfate to a saturation of 25% at 0°C with constant stirring. The suspension was incubated on ice for 1 h and then centrifuged at 27,000 × *g* for 30 min. The pellet was rinsed with deionized water and resuspended in 5 ml of resuspension buffer.

Photoaffinity cross-linking. A 20-μl reaction mixture containing partially purified $FtsZ_{Bb}$ (25% ammonium sulfate fraction from induced cells), 2 μg of bovine serum albumin, and 1 μM (3,000 Ci/mmol) either [α-^{32}P]GTP or [α-^{32}P]ATP in resuspension buffer was irradiated with UV light (Spectrolinker XL-1000; Spectronics Corp.) for 30 min on ice. The protein was precipitated with 10% trichloroacetic acid, washed once with cold acetone, and resuspended in 1× sample buffer.

PCR amplification. PCR amplifications were performed using a GeneAmp kit from Perkin-Elmer Cetus Corp., Norwalk, Conn. The 100-μl reaction mixture consisted of the template, forward and reverse primers, 1× amplification buffer, and a mixture of nucleotides (100 mM). The forward and reverse primers were complementary to the 5′ and 3′ ends of ORF-591 respectively. The amplifications were performed in a standard thermal cycler (Perkin-Elmer Cetus Corp.). PCR parameters consisted of 30 cycles of 94°C (1 min)–65°C (1 min)–72°C (1 min) followed by a 30-min extension period at 72°C. PCR products were purified by using the QIAQUICK PCR purification system from QIAGEN, Inc., Chatsworth, Calif., following the manufacturer's instructions.

Nucleotide sequence accession numbers. The nucleotide sequence of the 1,779-bp open reading frame of pIN1 has been deposited in the GenBank data base and has been given the accession number AF007266.

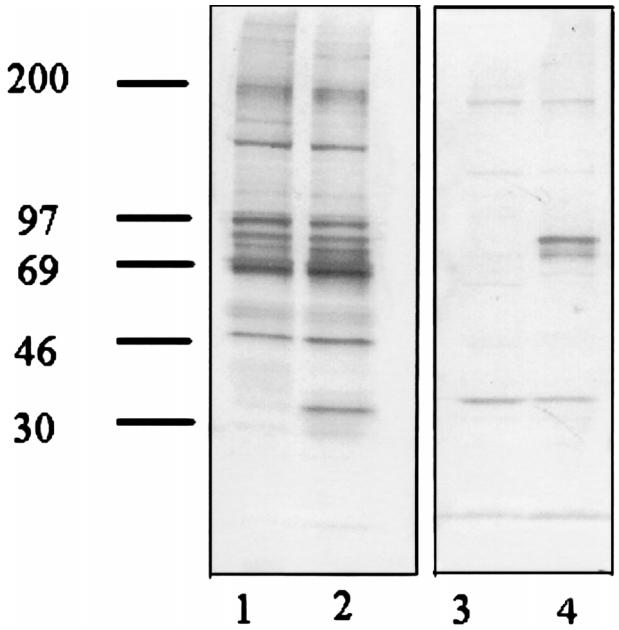

FIG. 1. SDS-PAGE and immunoblot of the clone expressing the 75-kDa antigen of *B. bacilliformis*. Lanes: 1 and 2, cell lysates from two strains of *B. bacilliformis*, KC583 and KC584; 3, XL-1*Blue* cells harboring pBluescript phagemid without insert; 4, XL-1*Blue* cells harboring recombinant clone pIN1 expressing the 75-kDa protein. Relative molecular masses (in kilodaltons) are indicated on the left.

RESULTS

Library screening. Approximately 50,000 recombinant lambda phage from the *B. bacilliformis* genomic DNA library were screened with human serum for expression of *B. bacilliformis*-specific antigens. Eight of the intensely reacting clones were plaque purified for further analysis. Seven of the clones expressed antigens that comigrated with antigenic proteins from cell preparations of the two strains of *B. bacilliformis*, KC584 and KC583. Two of these expressed an antigen of *B. bacilliformis* with an apparent molecular mass of 75 kDa (Fig. 1). One of them, designated pIN1, was used for nucleic acid sequencing.

DNA sequence analysis. The nucleotide sequence of the 2,441-bp insert of pIN1 revealed a 1,779-bp open reading frame capable of encoding a 591-amino-acid protein (ORF-591, Fig. 2). ORF-591 is oriented in the same direction as the *lacZ* promoter on the pBluescript cloning vector and extends from an AUG translational start codon at nucleotide 191 to the UGA stop codon at nucleotide 1969. Located about 8 nucleotides upstream of the start codon is a sequence (AGGAA GG) that is highly homologous to the consensus ribosome binding site for *E. coli* and other prokaryotes (30). ORF-591 uses a high percentage of preferred *B. bacilliformis* codons, as

FIG. 2. DNA sequence of the 2,441-bp insert of pIN1. The deduced amino acid sequence of the 1,779-bp open reading frame expressing the 75-kDa protein is shown in single letter code. The predicted ribosome binding site is highlighted and underlined. Amino acids identified by N-terminal sequencing are marked by asterisks.

revealed by comparisons with other characterized *B. bacilliformis* genes (21). pKKORF, a subclone of pIN1 that encodes ORF-591 on a 1,800-bp segment was found to express the 75-kDa antigen with high efficiency (data not shown) N-terminal sequence analysis of the 75-kDa protein produced by the cells harboring pKKORF revealed an amino acid sequence identical to that predicted for ORF-591 (Fig. 2).

Analysis of the protein sequence. The amino acid sequence specified by ORF-591 codes for an acidic protein with a predicted molecular mass of 63.4 kDa and an isoelectric point of 4.94. A search through the protein database Swiss-Prot revealed ORF-591 to be highly homologous to the FtsZ family of proteins, exhibiting levels of identity ranging from 20 to 69% (Table 1). The FtsZ protein, an essential component of the cell division process, has been reported for both gram-positive and gram-negative bacteria (5, 6). Alignment of the amino acid sequence of *B. bacilliformis* ORF-591 with the sequences of FtsZ proteins from *Rhizobium melliloti*, *E. coli*, *Bacillus subtilis*, *Micrococcus luteus*, *Streptomyces coelicolor*, *Staphylococcus aureus*, and *Wolbachia* species (Fig. 3) reveals a striking conservation of sequence, particularly in the N-terminal half of ORF-591 (residues 1 to 320). This region has been reported to contain all of the functionally important domains in the *E. coli* protein, including a glycine-rich GTP/GDP binding pocket (8, 24); two other glycine-rich sequences implicated in GTPase activity (7, 25); and a proposed magnesium-binding loop (25). A comparison of the amino acid sequence of the N-terminal domain of the *B. bacilliformis* FtsZ homolog with the sequences of other FtsZ proteins reveals sequence identity ranging from 45% in $FtsZ_{Ml}$ to 91% in $FtsZ_{Rm1}$ (Table 1).

TABLE 1. Comparison of homology between the amino acid sequence of the 75-kDa antigen of *B. bacilliformis* and previously characterized FtsZ proteins[a]

Protein	% Match	Over (amino acids)	% N terminus match	% C terminus match
RM1	55.1	606	91	46
RM2	44	345	80	
EC	47	353	60	
BS	36	400	60	
Wsp	69.3	332	81	
SA	37	390	60	
SC	40	399	57	
ML	20	170	45	
BBg	63.2	87	NA	

[a] Percent matches and lengths of homologous regions are indicated. Only identical amino acids are used in the comparison. The N-terminal domain is defined as the first 321 amino acids of the 75-kDa antigen, while the C-terminal domain is defined as the C-terminal 270 amino acids. RM1, *R. melliloti* (homolog 1); RM2, *R. melliloti* (homolog 2); EC, *E. coli*; Wsp, *Wolbachia* species; BS, *B. subtilis*; SA, *S. aureus*; SC, *S. coelicolor*; ML, *M. luteus*; Bbg, *Borrelia burgdorferi*; NA, entire sequence not available.

Relatedness to the *R. melliloti* FtsZ homolog, $FtsZ_{Rm1}$. With respect to size and overall homology, $FtsZ_{Bb}$ is most similar to the *R. melliloti* FtsZ homolog, $FtsZ_{Rm1}$. Like $FtsZ_{Rm1}$, $FtsZ_{Bb}$ is about twice as large as the other characterized FtsZ proteins. $FtsZ_{Bb}$ and $FtsZ_{Rm1}$ have 591 and 590 amino acids, respectively, while all other FtsZ proteins have less than 400 amino acids. $FtsZ_{Bb}$ and $FtsZ_{Rm1}$ have an extra 255 and 256 amino acids, respectively, at the C terminus that are not present in the other proteins. The homology of $FtsZ_{Bb}$ with the other FtsZ proteins diminishes dramatically around amino acid 300, which coincidentally is close to the C terminus of the smaller FtsZ proteins. The striking homology between $FtsZ_{Bb}$ and $FtsZ_{Rm1}$ (91%) within the N-terminal 300 amino acids falls to 46% for the C-terminal 256 amino acids. A conserved region is apparent at the extreme C termini of the two proteins, where a short stretch of around 14 residues exhibits sequence identity approaching 100%. This short conserved stretch of amino acids is also present at the C-terminal ends of the other FtsZ proteins, with the exception of $FtsZ_{Ml}$ and $FtsZ_{Rm2}$. Although the function of this region has not yet been elucidated, its high degree

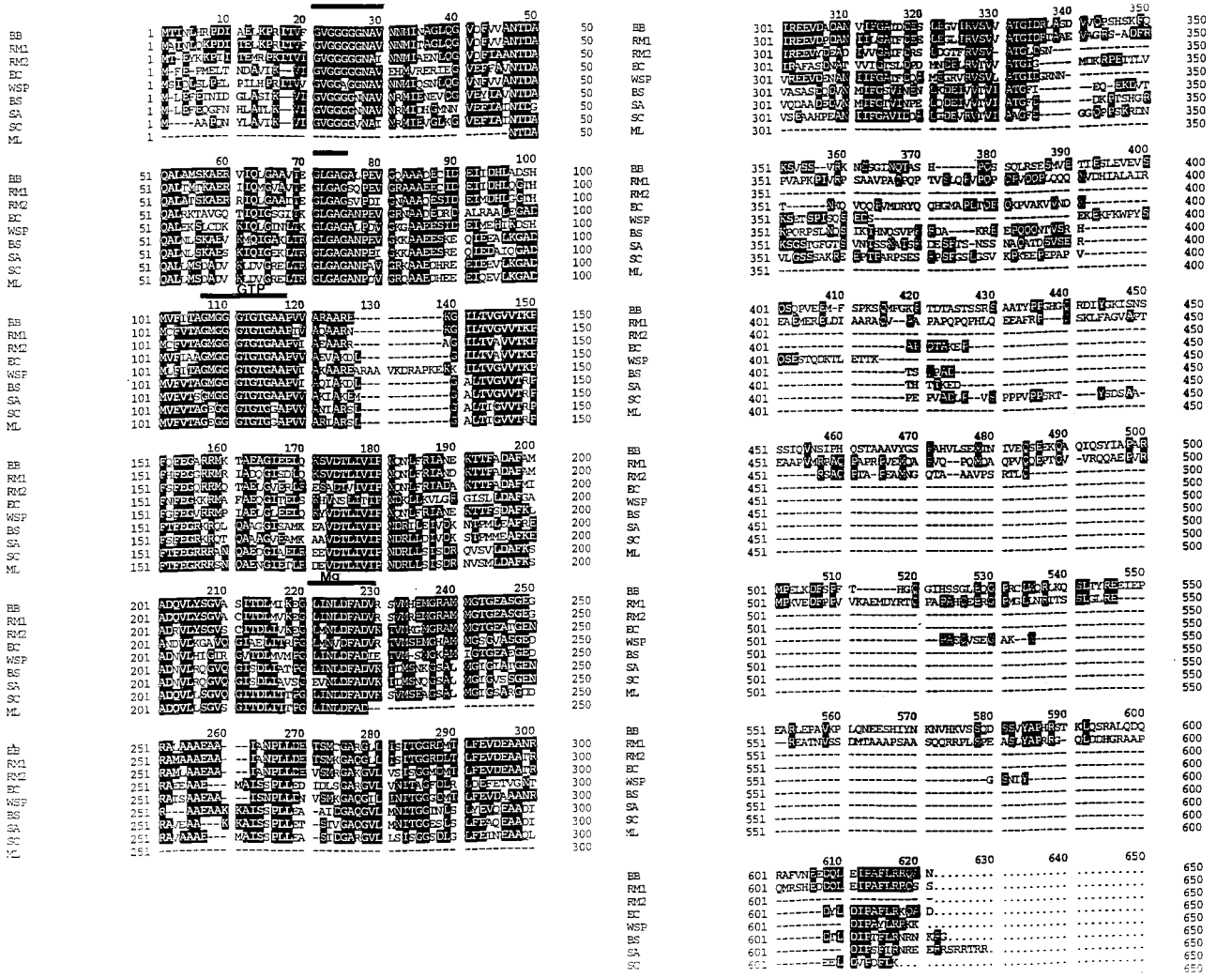

FIG. 3. Alignment of the amino acid sequence of the 75-kDa antigen of *B. bacilliformis* (BB) with sequences of FtsZ homologs from *R. melliloti* (RM1 and RM2), *E. coli* (EC), *Wolbachia* species (WSP), *B. subtilis* (BS), *S. aureus* (SA), *S. coelicolor* (SC), and *M. luteus* (ML). Regions of similarity are highlighted, and functionally important domains are overlined.

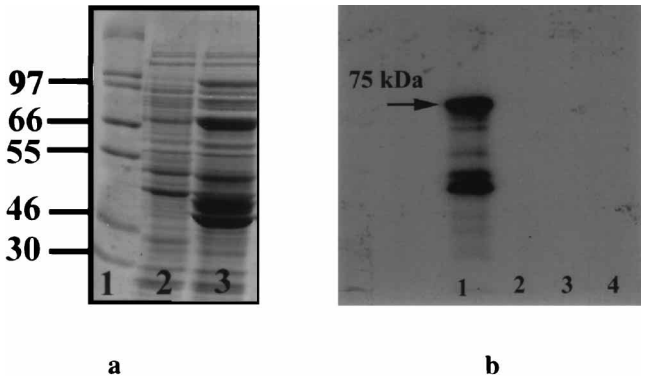

FIG. 4. Photoaffinity cross-linking of [α-^{32}P]GTP to the 75-kDa antigen. (a) Coomassie blue-stained gel of 10 to 20% ammonium sulfate fractions from cells expressing the 75-kDa antigen. Lanes: 1, molecular mass markers (in kilodaltons); 2 and 3, fractions from uninduced and induced cells, respectively. Lanes 1 and 2, 10 to 20% ammonium sulfate fractions from cells expressing the 75-kDa antigen under induced (lane 1) and uninduced (lane 2) conditions, cross-linked to [α-^{32}P]GTP. Lanes 3 and 4, fractions from lanes 1 and 2 cross-linked to [α-^{32}P]ATP.

of conservation both in the smaller and larger FtsZ proteins raises the possibility that it is important for FtsZ activity.

The molecular mass of $FtsZ_{Bb}$ as estimated by SDS-PAGE (75 kDa) is about 12 kDa more than the molecular mass predicted from the open reading frame (63 kDa). A slower migration pattern on SDS-PAGE has also been observed with $FtsZ_{Rm1}$. In this case, the aberrant migration was attributed to the acidic nature of the protein or an unusual folding pattern (17). One of these factors may also be the cause of the slower migration of $FtsZ_{Bb}$.

Interaction of $FtsZ_{Bb}$ with GTP. The conservation of a glycine-rich GTP/GDP binding pocket in $FtsZ_{Bb}$ suggests that the *Bartonella* protein, like other FtsZ homologs (8, 16, 24), plays a role as a GTP binding protein. We studied the GTP binding capability of $FtsZ_{Bb}$ by using photoaffinity cross-linking. A mixture consisting of a 25% ammonium sulfate fraction containing the protein and [α-^{32}P]GTP was exposed to UV light and analyzed by SDS-PAGE (Fig. 4b). $FtsZ_{Bb}$ was found to cross-link GTP with high efficiency (lane 1). The lack of apparent cross-linking to [α-^{32}P]ATP (lane 4) suggests that the binding is specific for GTP.

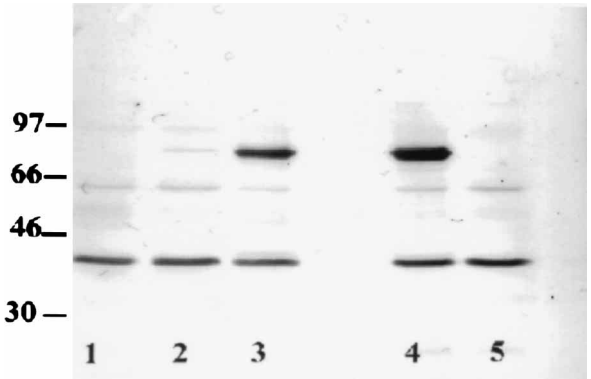

FIG. 5. Immunoreactivity of the 75-kDa antigen with *E. coli* FtsZ antiserum. Lanes 3 and 4 represent the 75-kDa protein expressed by cells harboring pIN1 and pKKORF, respectively, under induced conditions. Lanes 2 and 5 are the same cells under uninduced conditions. Lane 1 represents XL-1*Blue* cells harboring the pBluescript phagemid without an insert.

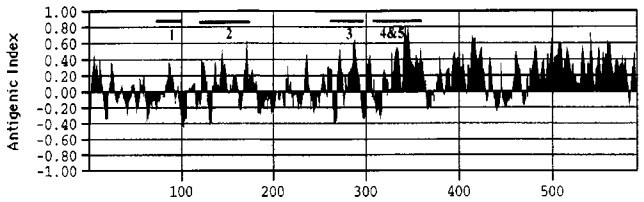

FIG. 6. Antigenicity profile of the *B. bacilliformis* FtsZ homolog. Regions above baseline are predicted antigenic determinants. Prediction was made using the software program MacVector. The algorithm combines the information from hydrophilicity, surface probability, and backbone flexibility with the secondary structure predictions of Chou-Fasman and Robson Garnier. Epitopes mapped in the *E. coli* FtsZ homolog are overlined.

Immunoreactivity of $FtsZ_{Bb}$. Western blot analysis indicates that the 75-kDa proteins expressed from pIN1 and pKKORF react strongly with antiserum raised against purified *E. coli* FtsZ (Fig. 5, lanes 3 and 4). This finding is not unexpected in view of the strong sequence conservation between $FtsZ_{Ec}$ and the N-terminal region of $FtsZ_{Bb}$. Moreover, all of the epitopes that have been mapped in $FtsZ_{Ec}$ are conserved in $FtsZ_{Bb}$ (Fig. 6). These extracts contain a second protein of approximately 40 kDa that is also detected by the anti-$FtsZ_{Ec}$ antibodies. The size of this protein and the fact that it is observed in control cells lacking ORF-591 suggest that it corresponds to the FtsZ homolog encoded by *E. coli* ($FtsZ_{Ec}$). Surprisingly, the anti-*Bartonella* antiserum failed to detect a protein of this size in the *E. coli* strain used for the construction of the *B. bacilliformis* gene library (Fig. 1), even though these cells would be expected to contain endogenous $FtsZ_{Ec}$. This apparent lack of reactivity on the part of $FtsZ_{Ec}$ with the *Bartonella* antiserum suggests that the epitopes responsible for the antigenicity of $FtsZ_{Bb}$ in the human serum are not shared by its *E. coli* counterpart.

A fortuitous observation has led us to theorize that the anti-FtsZ antibodies generated during infection with *B. bacilliformis* are directed predominantly against epitopes located in the C-terminal region of the protein. We have observed that, upon storage, the 75-kDa protein is broken down into four smaller immunoreactive fragments. Each of these fragments is immunoreactive only with one type of antiserum: three can be detected by the anti-*Bartonella* human serum but are not recognized by anti-$FtsZ_{Ec}$ antibodies, while a fourth reacts solely with antibodies raised against the *E. coli* protein (data not shown). N-terminal sequence analysis of two of the anti-*Bartonella* immunoreactive fragments indicates that they are derived from the C-terminal half of $FtsZ_{Bb}$, while the fragment reacting only with the anti-$FtsZ_{Ec}$ serum is derived from the N-terminal end (Table 2). These observations, like the results presented above, suggest that the epitopes on $FtsZ_{Bb}$ recognized by the human antiserum are not shared by the *E. coli* protein and, further, localize them to the C-terminal, nonhomologous region of the protein. The photoaffinity cross-linking experiments also reveal a GTP binding protein of approxi-

TABLE 2. N-terminal amino acid sequences of proteolytic degradation products of $FtsZ_{Bb}$

Fragment	Reactivity	N-terminal sequence	Predicted molecular mass (kDa)	Observed molecular mass (kDa)	Proposed location
1	anti-*Bartonella*	337-KSVSSVRKNDS	27.17	32.0	C terminus
2	anti-*Bartonella*	344-KNDSGINQTA	24.94	32.0	C terminus
3	anti-*E. coli*	1-MTINLHRPDIA	36.96	43.0	N terminus

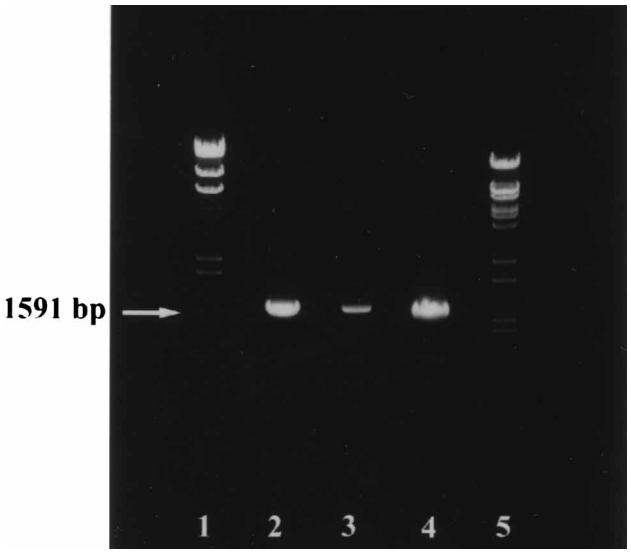

FIG. 7. PCR amplification of the 75-kDa-antigen gene from *B. bacilliformis* and *B. henselae* genomes. Lanes 2, 3, and 4, amplification products obtained using *B. bacilliformis* and *B. henselae* genomic DNA and pIN1 as templates; lanes 1 and 5, molecular weight markers (λ*Hin*dIII [lane 1] and λ*Bst*EII [lane 5]).

mately 43 kDa (Fig. 4). Since it is observed only under conditions where expression of $FtsZ_{Bb}$ is induced from pKKORF (Fig. 4b, lanes 1 and 2), it is possible that the 43-kDa GTP binding protein corresponds to the N-terminal degradation fragment of this size that was observed upon storage of $FtsZ_{Bb}$. If so, this observation provides evidence supporting the assignment of the GTP binding region to the N-terminal domain of the *Bartonella* protein.

PCR amplification of the FtsZ gene sequence from *B. henselae* DNA. To determine if the closely related *B. henselae* had an FtsZ gene homologous to that of *B. bacilliformis*, we subjected a sample of *B. henselae* genomic DNA to PCR amplification conditions using primers complementary to regions near the 5′ and 3′ ends of the *B. bacilliformis* FtsZ gene. The sequences of the primers were as follows: forward primer, 5′ GCTTGGCGCAGCAGTGA 3′; reverse primer, 5′ ACGTA AAAATGCTGGTATTTC 3′. An amplification product was consistently detected with *B. henselae* DNA, with a size (1,591 bp) that corresponds closely to that of the amplification product from the *B. bacilliformis* genomic DNA template (Fig. 7).

DISCUSSION

In this report we describe the construction of a *B. bacilliformis* genomic library using *B. bacilliformis* strain KC584 and the isolation of a clone that expresses a 75-kDa antigen of *B. bacilliformis*. A 75-kDa antigen was previously described as one of the two main antigens of *B. bacilliformis* since it consistently showed strong reactivity in immunoassays (13). Additionally, it was found to be one of the three antigens that could detect persisting antibodies in patients. The amino acid sequence of the 75-kDa protein encoded by ORF-591 bears a very high degree of homology to the FtsZ family of proteins. We suggest that the 75-kDa antigen is an FtsZ homolog of *B. bacilliformis.*

Studies with *E. coli* have shown that FtsZ plays a central role in bacterial cell division, since it initiates the process by forming a cytokinetic ring at the division site (16). This leads to the circumferential invagination of the cytoplasmic membrane and cell wall, followed by formation of the division septum. The ring formation is facilitated by the ability of FtsZ monomers to self-assemble, a process that is dependent on the GTPase function of FtsZ (9, 22). The GTP binding domain of FtsZ (GGG TGTG) bears a remarkable similarity to the GTP binding domains of the eukaryotic tubulins (GGGTGSG), which suggests that FtsZ molecules polymerize in a manner analogous to the tubulins.

The central role of FtsZ in cell division is reflected in its strong conservation among eubacteria. Homologs of FtsZ, all with a high degree of sequence similarity, have been reported in several bacteria (2, 5, 12, 17–19). The alignment of the amino acid sequence of $FtsZ_{Bb}$ with the sequences of previously characterized FtsZ proteins shows several blocks of sequences in the N-terminal domain to be conserved (Fig. 3). The glycine-rich GTP binding pocket and other regions proposed to be involved in the GTPase activity of FtsZ (^{21}GVGG, ^{71}GLGAG, and ^{223}NL/VDFAD) are nearly 100% conserved in $FtsZ_{Bb}$. Using photoaffinity cross-linking we have shown that $FtsZ_{Bb}$ binds GTP very efficiently and that the binding is specific for GTP. The strong conservation of functional domains and epitopes suggests that with respect to its FtsZ function, the *B. bacilliformis* homolog of FtsZ may act in a manner analogous to other characterized FtsZ proteins.

The feature that distinguishes $FtsZ_{Bb}$ from most of the other FtsZ proteins characterized to date is the presence of a 256-residue tail at the C terminus. This is a property that it shares only with the *R. melliloti* FtsZ homolog, $FtsZ_{Rm1}$. The function of this C-terminal domain is unknown. All of the domains identified to be necessary for the activity of FtsZ in cell division (17) are located in the N-terminal 300 residues of both $FtsZ_{Bb}$ and $FtsZ_{Rm1}$. The C-terminal tail may therefore play a role within the cell that has not been previously associated with FtsZ proteins. In $FtsZ_{Rm1}$, this region consists of a high proportion of proline and glutamine repeats, which were predicted to form a stacked β-sheet secondary structure (17). The C-terminal tail of $FtsZ_{Bb}$ has a high content of serine and glutamine residues, making it very hydrophilic. It is therefore possible that this domain lies in an aqueous environment such as the outside of the protein or the outside of the bacterial cell.

Analysis of the amino acid sequence of $FtsZ_{Bb}$ predicts the conservation of all epitopes that have been mapped in its *E. coli* counterpart (33; Fig. 6). This expectation is confirmed by the strong reactivity exhibited by $FtsZ_{Bb}$ with the *E. coli* FtsZ antiserum (Fig. 5). On the other hand, we were unable to detect *E. coli* FtsZ with anti-*Bartonella* antiserum. This observation, plus the apparent nonreactivity of the N-terminal fragment of $FtsZ_{Bb}$ with the anti-*Bartonella* antiserum, suggests that the epitopes recognized by the human serum lie within the unique C-terminal region of $FtsZ_{Bb}$. Theoretical predictions based on hydrophilicity, surface probability, and flexibility indicate that this region has a high proportion of antigenic sites (Fig. 6). Taken together, our findings suggest that the C-terminal domain of $FtsZ_{Bb}$ is the region that elicits the predominant immunogenic response during the infectious process.

It is not known whether *B. bacilliformis* undergoes lysis in the infected host. However, if the anti-*Bartonella* antibodies in the human serum used for these studies are directed against intact bacteria, it is possible that at least part of the 75-kDa antigen, most likely its C-terminal domain, is exposed on the surface of the cell. If so, the subcellular location of $FtsZ_{Bb}$ would differ from that of most organisms studied to date (3), for which FtsZ has been reported to be located primarily in the cytoplasm. Earlier reports indicate the presence of an antigen the size of $FtsZ_{Bb}$ (75 kDa) in membrane preparations of *B. bacilliformis* (13, 20).

The unusual size of $FtsZ_{Bb}$ raises the possibility that the

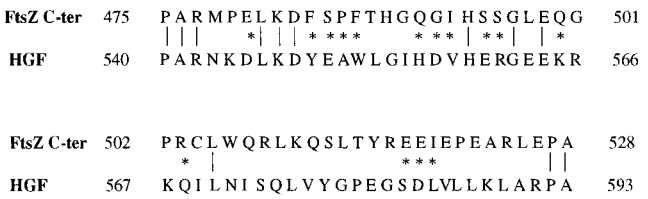

FIG. 8. Comparison of homologous regions of $FtsZ_{Bb}$ (FtsZ C-ter) and rat HGF precursor. Identical residues are indicated by lines, and equivalent amino acids are indicated by asterisks.

75-kDa antigen may not actually be involved in the ring structure normally associated with FtsZ function and that this role is carried out by the product of another, as yet undetected, *ftsZ* gene that lacks the C-terminal coding region. A second FtsZ homolog ($FtsZ_{Rm2}$) that lacks the C-terminal extension was identified in *R. melliloti* (18); however, this homolog was found to be nonessential for viability. Southern hybridization of the *B. bacilliformis* genome to the cloned $ftsZ_{Bb}$ gene did not reveal any restriction fragments that could be attributed to a second *ftsZ* gene (data not shown). Thus, we have no evidence at this time to indicate that a second FtsZ homolog is encoded on *B. bacilliformis* DNA.

It is possible that a fraction of the newly synthesized 75-kDa molecules is cleaved following synthesis, thereby producing a subset of proteolytic fragments that are capable of forming a cell division structure analogous to the ring structures found in other bacteria. It is worth noting that a short segment within the C-terminal domain of $FtsZ_{Bb}$ is homologous to the light chain of the rat hepatocyte growth factor (HGF), the cleavage of which is essential for activation of the protein (31) (Fig. 8). Coincidentally, the amino acid sequence that $FtsZ_{Bb}$ shares with HGF has been reported to be homologous to the serine protease domain of plasmin.

The amplification of a DNA segment from *B. henselae* with primers specific for *B. bacilliformis ftsZ* suggests that *B. henselae* also contains a large FtsZ homolog. Moreover, when extracts of *B. henselae* are reacted with *E. coli* FtsZ antiserum, an immunoreactive band of approximately 75 kDa is detected (data not shown). The discovery of FtsZ homologs that are twice the size of the conventional FtsZ proteins in these *Bartonella* strains raises the possibility that large FtsZ proteins form a distinct subgroup of FtsZ homologs with the potential for dual or multiple functions. A large FtsZ protein in the plant pathogen *Agrobacterium tumefaciens* has also been reported (5). Interestingly, all of these bacteria belong to the α-2 subgroup of the class *Proteobacteria* (23). Coincidentally, members of this subgroup are also known to interact closely with eukaryotic cells. In the case of *Bartonella*, this interaction results in the hemangioma-like lesions of BA. Thus, it appears that some component of the infection process induces the proliferation of eukaryotic cells. Recently, we have observed that endothelial cells preferentially bind partially purified $FtsZ_{Bb}$ (unpublished observations). Characterization of this interaction may enable us to determine the precise role of this FtsZ homolog during *B. bacilliformis* infection.

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